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# LIGHT TRANSMISSION AND SPECTRAL DISTRIBUTION THROUGH EPI- AND ENDOZOIC ALGAL LAYERS IN THE BRAIN CORAL, *FAVIA*[1]

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Two species of algae were living symbiotically with a hard brain coral, *Favia*, harvested in the environs of the Flinders Island Group on the Great Barrier Reef in Australia. One of the algae has the microscopic appearance and pigment composition of dinoflagellates (Halldal, 1968, Jeffrey and Haxo, 1968) and it contributes the dark brown color to the coral tissues which form a surface layer over the colony. This alga resembles *Symbiodinium microadriaticum* Freudenthal. Green algae lived inside the spherical coral forming another colored layer (Jeffrey, 1968), and there was an intermediate pale green (nearly white) layer between these brown and green layers. The green algae seemed to be of mixed genera and species, most of them probably belonging to *Ostreobium Reineckei* Bornet within the order Siphonales. The present paper describes the *in vivo* absorption spectra of these algal layers as well as the spectral distribution.

## Experimental Methods

The samples of *Favia pallida* Dana were harvested in the environs of the Flinders Island Group, and were kept in running sea-water aquaria on the open deck of the research vessel. The spectral data presented in this paper were observed for the sample which was 12 cm in diameter and 8.5 cm in thickness (Fig. 1 left). The coral colony had a brown layer of 4 mm thick, a green layer of 2 mm and an intermediate layer of 9 mm between them. A square piece of about $4.2 \times 4.2$ cm$^2$ in surface area was cut out of this sample with an electric sawing machine, and the white lime below the green layer was ground off to obtain a piece with the three layers such as shown on the right side of Figure 1. The piece with the three layers was further cut or ground to obtain a sample of a single or double layer according to experimental requirements.

Spectroscopic measurements were carried out on the research vessel with a Shimadzu Multipurpose recording spectrophotometer model MPS–50. This spectrophotometer designed by Shibata, one of the authors, had two photomultipliers with the end-on type of photocathode, one for the sample and the other for the reference, and was suitable for the measurements of translucent and dark pieces of corals for the following reasons. a) The double detector system makes it

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possible to read a high absorbance value accurately because of the absence of interaction (cross talk) between the separate electric channels from the two photomultipliers. In ordinary recording spectrophotometers with a single photomultiplier, the cross talk between alternative sample and reference signals (photocurrents) from the single detector introduces systematic errors to the absorbance reading when the reading is high. b) With the spectrophotometer, absorbance can be read up to 3 by electric amplification, and the use of a light attenuator for the reference beam together with this electric amplification enables us to measure an absorbance value as high as 6. c) The end-on photocathode captures all of the light transmitted through a piece of coral placed close to the photocathode, so that the reading is an absolute value of semi-integral attenuance (Shibata, 1958 and Shibata, 1959) necessary to calculate the transmittance through such translucent samples (Shibata, Benson and Calvin, 1954). This contrasts with the measurements with the side-on type of photomultipliers commonly used. The small photocathode inside the tube of the side-on type captures only a small fraction of the

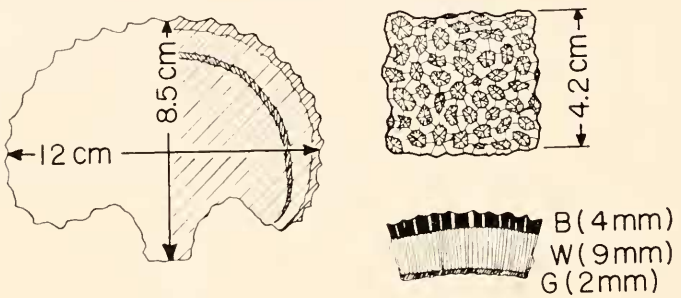


FIGURE 1. The sample of *Favia*; the dimensions of a vertical radial section (the left figure) and a piece cut for spectroscopic measurement (the right figures) as seen from above (upper figure) and again in vertical section (lower figure). B, W and G stand for the brown, the intermediate white and the green layers, respectively.

diffuse transmitted light, and the fraction varies, depending on the optical geometry of the instrument and the angular distribution of the diffuse transmitted light (Shibata, 1959). When such a detector is used in making measurements on translucent samples, quasi-attenuance (Shibata, 1959) is measured rather than absorbance or semi-integral attenuance (Shibata, 1959). The light transmitted through a heterogeneous translucent sample is generally composed of different kinds of light with different angular distributions of intensity. A typical example is the light transmitted through a cell suspension which is composed of completely parallel light transmitted through the suspending medium and diffuse light transmitted through the cells in suspension. The measurement at a distance from a heterogeneous sample captures different fractions of these different light fluxes, so that the spectrum thus observed in terms of quasi-attenuance is greatly distorted as demonstrated previously (Shibata, Benson and Calvin, 1954, Shibata, 1957, and Shibata, 1958). d) The photocathode of the end-on photomultiplier, Shimadzu R–236, developed for this spectrophotometer is more red-sensitive than ordinary photocathodes. A high resolution was thereby obtained in the spectral region of

the red bands of chlorophylls without sacrificing much sensitivity in other spectral regions.

Fluorescence action (or excitation) spectra were observed with the same spectrophotometer, using the fluorescence attachment model I. A red filter, which cut the light below 660 mμ, was used for the measurement of the red fluorescent light of chlorophyll *a*.

## Results and Discussion

The absorption spectrum of the brown layer of 3 mm thick is shown by curve A in Figure 2, where the absorbance value at 800 mμ is taken to be zero; the spectrum in absolute units is shown in Figure 3. The spectrum shows the red band of chlorophyll *a* at 678 mμ and its Soret band around 430 mμ, both being in agreement with the maximum wavelengths obtained previously for intact green leaves and algae (Shibata, Benson and Calvin, 1954, Shibata, 1957 and Shibata, 1958). In addition to these maxima, the spectrum shows maxima and shoulders at 635, 620, 590, 540, 500, 470, and 430 mμ. The two bands at 635 and 590 mμ are interpreted as the bands of chlorophyll *c* (Jeffrey, 1963, Jeffrey and Allen, 1964, Jeffry and Haxo, 1968, Jeffry and Shibata, 1969, and Shibata, Benson and Calvin, 1954), and the band at 620 mμ may be the second red band of chlorophyll *a*. A

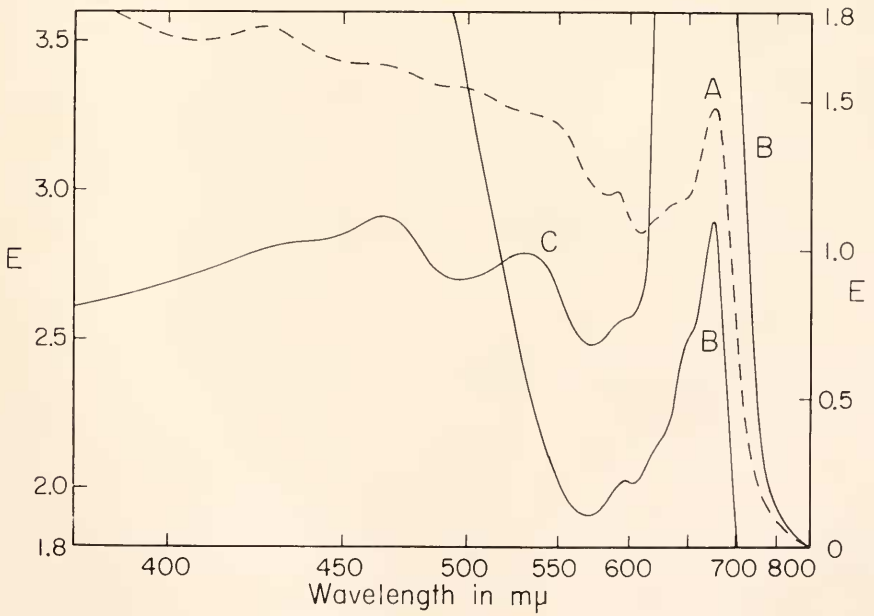


Figure 2. The *in vivo* absorption spectra of the brown layer of 4 mm thick (curve A) and the green layer of 2 mm thick (curve B), and the fluorescence action spectrum of the brown layer (curve C). The absorbance value, $E$ (more correctly speaking, the semi-integral attenuance, ${}_{p}E_{t}$; Shibata, 1959) at 800 mμ for curves A and B are taken to be zero, and the action spectrum is shown in arbiturary units. The scale on the left side of the figure is for curve B above $E = 1.8$, and that on the right side is for curves A and B below $E = 1.8$.

characteristic round band at 540 mμ in this spectrum may be ascribed to peridinin (Jeffrey and Haxo, 1968), and the wavelength is in agreement with that of a peak found in the photosynthetic action spectrum of the same alga taken from the brown layer (Halldal, 1968). The bands at 430 and 470 mμ may be due to chlorophylls *a* and *c*, respectively, and a band at 500 mμ may be a composite of carotenoid bands (Jeffrey and Haxo, 1968). The photosynthetic activity versus light intensity was measured by Halldal (1968) for the same alga. The photosynthetic activity curve thus measured with the light at 440 mμ indicated a linear relationship below the intensity 1500 erg/cm² sec, saturation at 95,000 erg/cm² sec and no inhibition of activity at 225,000 erg/cm² sec which is approximately equal to the sun light energy available for photosynthesis below 700 mμ on a sunny day on the earth.

Curve C in the same figure shows the fluorescence action (excitation) spectrum obtained for the alga taken from the brown layer. The two distinct bands at 465 and 530 mμ indicate contribution of both peridinin and chlorophyll *c* to the fluorescence of chlorophyll *a*. This implies a transfer of energy from these pigments to chlorophyll *a*. The band at 500 mμ in the *in vivo* absorption spectrum is lacking in the action spectrum. This indicates the transfer of little or no energy from the carotenoids having an absorption maximum at this wavelength.

The spectrum of the green layer of 2 mm thick is shown by curve B which indicates the absorption characteristics of the green algae; red bands of chlorophyll *a* at 678 mμ and 620 mμ and a shoulder of chlorophyll *b* at 650 mμ. The shoulder of chlorophyll *b* at 650 mμ is more distinct than in *in vivo* spectra of common green algae such as *Chlorella* and *Scenedesmus*. The higher content of chlorophyll *b* in the green algae was reflected in the photosynthetic action spectrum observed by Halldal (1968), and agrees with the observation by Jeffrey (1968) that the content of chlorophyll *b* is about $\frac{2}{3}$ of the chlorophyll *a* content. The *a*/*b* ratios determined by a new precise method for several species of common plants and algae are very close to 3 (Ogawa and Shibata, 1965). The small peak at 595 mμ may be ascribed also to the high content of chlorophyll *b*. The spectrum in the Soret region shows a chlorophyll *a* band around 430 mμ and a round shoulder around 470 mμ as shown by curve B in Figure 3. Curves A and B in Figure 3 are the same spectra as curves A and B in Figure 2, respectively, but shown in absolute values ($E$) of semi-integral attenuance, the attenuance in terms of total transmitted light (Shibata, 1959). It is clear from these curves that the thinner green layer absorbs light more strongly than does the brown layer.

*Favia* has a brain-shaped surface with many ridges as shown on the right side of Figure 1. The dinoflagellates live in tissues of the coral between the ridges, and each dark brown area surrounded by ridges was 3 to 6 mm in diameter. When we looked at a lamp through a piece of the brown layer from its inside, we could recognize two kinds of light transmitted through the layer; brown dim light through the algal part between the ridges and nearly white light through the ridges. Therefore, the reading of attenuance of a single brown layer was considerably dependent on the area to be measured. On the other hand, the spectrum of a double layer composed of the brown and the intermediate layers was less sensitive to the area to be measured. This seemed to be due to the light-diffusing effect of the thick intermediate layer which will homogenize the two kinds of light transmitted through the heterogeneous brown layer. The spectrum of a piece of

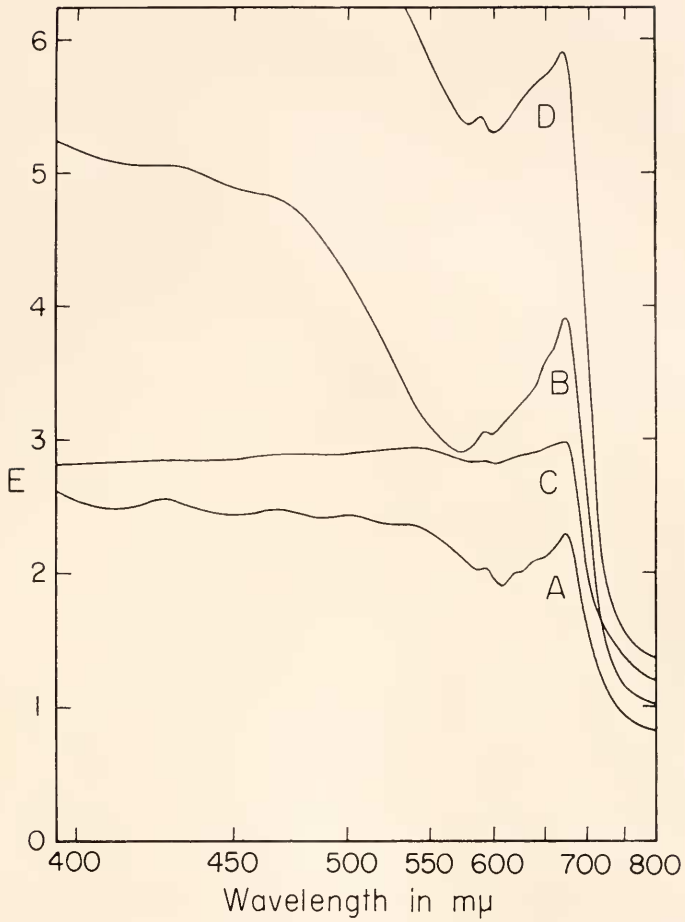


FIGURE 3. The *in vivo* absorption spectra in absolute units of semi-integral attenuance, ${}_pE_\lambda$, of a brown layer (curve A) of 4 mm thick, a green layer (curve B) of 2 mm thick, a double layer (curve C) composed of the brown and the intermediate white layers (13 mm in total thickness) and a triple layer (curve D) composed of the brown and the intermediate layers and one half (1 mm) in thickness of the green layer.

the double layer is shown by curve C in Figure 3, which indicates practically no change of attenuance below 678 mμ. This implies that the white light transmitted through the ridges predominates in intensity over the brown light transmitted through the algal part. The attenuance values read between 400 and 678 mμ ranged from 2.82 to 2.99 which corresponds to 0.10–0.15% in transmittance. The sun light intensity on the earth may be about 100,000 lux on a sunny day in the tropical area which is roughly 500,000 erg/cm² sec in terms of the energy available for photosynthesis below 700 mμ. The above attenuance values, therefore, indicate that the light falling on the upper surface of the green layer is 100–150 lux or 500–750 erg/cm² sec as the energy available for photosynthesis. Halldal (1968)

found that the alga taken from the top of the green layer shows linear response with intensity to 1000 erg/cm² sec at 440 mμ, saturation between 1000 and 1500 erg/cm² sec, and no photosynthetic inhibition at 5000 erg/cm² sec. The fact that the intensity on the upper surface of the green layer is slightly though not much lower than the saturation intensity suggests that the attenuation of light through the double layer is significant. The light below 700 mμ required for photosynthesis comes mostly through the ridges, and the ridges in combination with the intermediate white layer thus works as a gray filter for the green alga to bring about photosynthesis under an optimum dim light condition. The attenuance value on curve C drops steeply above 678 mμ. Above this wavelength, the light transmitted through the brown algal part between the ridges predominates over the light transmitted through the ridges, and this phenomenon seems to be responsible for the great drop of attenuance on curve C (Fig. 3). The attenuance values read at 700, 720, and 800 mμ on this curve were 2.23, 1.67 and 1.20.

Curve D in Figure 3 is the spectrum of a triple layer composed of the brown and white layers and about one half the thickness of the green layer. The attenuance value on the curve is 5 to 6 between 550 and 680 mμ and is higher than 6 below 550 mμ. This indicates almost complete absorption of the weak light by the green layer. The spectrum of a piece of a complete triple layer could not be observed directly because the attenuance was too high. The spectrum may, however, be calculated as the sum of curves B and C in Figure 3, since the effect of the multiple reflection between the green and the white layers may be assumed to be negligible when we have such a dark green layer on one side (Shibata, 1959). The attenuance values of the complete triple layer thus calculated at the Soret maximum, 430 mμ, at the minimum, 580 mμ, and at the red maximum, 678 mμ were roughly 8, 6 and 7, and those at 700, 720 and 800 mμ were 4, 5 and 2, respectively.

These data indicate that the light intensity in the middle of the green layer is roughly 0.5 to 5 erg/cm² sec between 550 and 680 mμ, and less than 0.5 erg/cm² sec below 550 mμ, and that the intensity reaching the bottom of the green layer is 0.005, 0.5, 0.05 and 5000 erg/cm² sec at 430, 580, 678 and 800 mμ, respectively. The photosynthetic activity observed by Halldal (1968) for the cells taken from the middle of the green layer showed linear response with intensity to about 100 erg/cm² sec, saturation between 100 and 700 erg/cm² sec, reduced activity between 700 and 1800 erg/cm² sec and photooxidation above this intensity. The low light intensity in the middle of the green layer allows such cells to grow without inhibition. On the other hand, the cells taken from the bottom of the green layer showed photooxidation at all intensities applied (Halldal, 1968), and the pigments extracted from the deep area contained decomposition products of chlorophylls, which suggests that the cells eventually become moribund with consequent decomposition of chlorophylls (Jeffrey, 1968). The very low light intensity reaching the bottom of the green layer is sufficient to cause the photooxidation observed *in vitro* by Halldal (1968). Another fact to be noted (Halldal, 1968) is that a shoulder appears at 720 mμ in the absorption spectrum when the green layer is kept in darkness. The formation of this band related to the high light transmission at 720 mμ, which is approximately 2%, has been discussed in his paper.

Coral colonies contract their polyps when removed from the sea, and the contraction exposes the ridges to sunlight while polyps expanded under the sea

cover the ridges. Therefore, the spectra observed in the present study for the contracting state may be different from those in the expanding state. The effect of this expansion will make the absorption bands higher and sharper as expected from the theory and experiments of the flattening effect (Duysens, 1956, and Itoh, Izawa and Shibata, 1963), which is the spectral change due to localization of pigments and is generally smaller in order than the artifact caused by measuring the quasi-attenuance of heterogeneous translucent samples by a common technique. The flattening effect in this case may be appreciable, but will not change the orders of the light intensities in corals discussed above.

## Summary

Two species of algae are living symbiotically with a hard brain coral, *Favia pallida;* a brown alga resembling *Symbiodinium microadriaticum* Freudenthal as a brown surface layer and green algae, most of them probably belonging to *Ostrebium Reineckei* Bornet, as a green layer with an intermediate white layer between them. The *in vivo* absorption spectra of these algal layers were observed with a new spectrophotometer suitable for the measurements of translucent dark pieces of corals, and light transmission through these layers of algae and its spectral distribution were calculated from the spectra. The spectrum of the top brown layer showed the band characteristics of dinoflagellates, and the *in vivo* bands of chlorophylls *a* and *c* and peridinin were identified. The bands of chlorophyll *c* and peridinin were also found in the fluorescence action spectrum which indicates energy transfer from these pigments to chlorophyll *a*. The spectrum of the third layer showed the band characteristics of green algae, and the light intensity and spectral distribution after transmission through the brown and the intermediate layers were found to be suitable for the green alga on the top of the green layer to bring about photosynthesis actively. On the other hand, the light intensity in the middle or at the bottom of the green layer was very low, being consistent with the fact that the pigments of the cells in these deep areas are easily photooxidized.

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